Remarks/Arguments:

These remarks accompany a request for continued examination and are responsive to the Advisory Action dated September 4, 2008.

Applicants acknowledge with thanks that the 102(a) rejections of claims 1-12 and the 102(b) rejections of claims 1, 3, 7, and 8 have been withdrawn.

Claims 1-12 stand rejected under Section 103(a) as unpatentable over Litt (US Pat. No. 6, 635,469) and Silva *J. Biol. Chem. 264*: 15863-15868, 1989). Applicants traverse these rejections for the following reasons.

"In determining the differences between the prior art and the claims, the question under 35 USC 103 is ... whether the claimed invention as a whole would have been obvious." MPEP Section 2141.02 (I). "Distilling an invention down to the 'gist' or 'thrust' of an invention disregards the requirement of analyzing the subject mater 'as a whole.'" Section 2141.02 (II).

Applicants' claimed method is directed to recovering native protein from a sample of *non-native protein aggregates* and includes steps of subjecting an aggregate sample that is *free of denaturants* to hydrostatic pressure to dissociate the aggregate, and then returning the disaggregated sample to ambient pressure *without cycling the pressure*, whereby the dissociated protein *refolds to native protein*.

The Office Action of June 11, 2008 states that the combination of the Litt and Silva references renders Applicants' claimed method obvious for the following reasons. Litt teaches "methods of recovering properly folded proteins from a sample comprising protein aggregates comprising (a) obtaining a sample comprising protein aggregates ... without any urea or other reducing agents, (b) subjecting the sample to elevated hydrostatic pressure to partially dissociate proteins from aggregates, (c) returning the sample to ambient pressure to allow refolding of the protein." The Office Action also states that Litt does teach cycling between high pressure and ambient pressure, that Litt teaches reversible dissociation and aggregates in inclusion bodies, and that Litt teaches that chaotropic agents are optional (Office Action, pages 5-6). The Office Action states that Silva teaches "methods of recovering native protein from a sample comprising protein aggregates comprising (a) obtaining a sample comprising protein

aggregates which is free of denaturing agent, (b) subjecting the sample to elevated hydrostatic pressure causing dissociation, and (c) returning the sample to ambient pressure without repeated cycles," (Office Action, page 6).

Therefore, according to the Office action, one of ordinary skill in the art would have been motivated to combine the methods of Litt and Silva to arrive at Applicants' method because Silva teaches that hydrostatic pressure can be used without cycling to dissociate large protein aggregates without denaturing the protein (OA pages 6-7). The Office Action also states that one of ordinary skill in the art would have had a reasonable expectation of success in combining the methods of Litt and Silva, because Litt teaches cycling between pressures to dissociate protein aggregates and Silva teaches dissociation of aggregates without cycling (OA, page 7). However, in this analysis, the Office Action fails to consider Applicants' invention "as a whole."

It is important to note the distinction between the types of "aggregates" discussed by Litt vs. Silva and the distinction between the methods described by Litt for the dissociation of protein aggregates versus the refolding of proteins. Litt teaches that *dissociation* of *native protein complexes* (natural binding partners such as antigen-antibody or enzyme-substrate) can be obtained without denaturation. However, for *non-native aggregate proteins*, such as those found in inclusion bodies and those to which Applicants' method is directed, Litt teaches that it is necessary to use pressure cycling and a solid support, such as an exchange resin, to cause the proteins to refold, and *to denature the protein* first using very high pressure or a denaturant (Litt, Col. 17, line 45 through Col. 18 line 31). In contrast, Applicants claim a method for refolding *non-denatured* proteins from *non-native protein aggregates without pressure cycling*.

Litt specifically teaches *refolding of denatured proteins from non-native protein aggregates*. Aggregates of *denatured* protein are subjected to elevated pressure to form *denatured* protein chains and subsequently the pressure is *rapidly cycled* to cause *denatured* polypeptides to fold properly (Col. 9, lines 17-25). Refolding is also discussed by Litt beginning at Col. 17, line 62 as described above. In Col. 18, Litt first discusses the use of pressure cycling in an ion-exchange system wherein the proteins must bind to a resin in order to refold. At Col. 18, lines 21-25, Litt teaches that cross-linked aggregated proteins can be refolded using pressure and a buffer to *denature* the protein molecules. Protein *refolding* is only discussed in Litt in these two cited sections. No examples, either with or without denaturing or pressure cycling, are presented by Litt that demonstrate protein *refolding*. Litt's examples are directed

either to using pressure to enhance binding of a protein to a natural binding partner or to dissociate native protein complexes, such as antibody-antigen complexes, which are native, multi-subunit protein complexes and not non-native protein aggregates.

Silva describes the effects of pressure on hemoglobin, a *native* oligomeric protein having several subunits. Applicants have specifically excluded native proteins comprising multiple subunits from their definition of "aggregate." In paragraph 0030 of the specification, "aggregate" is defined as follows: "Specifically, the term 'protein aggregate' is not intended to include the normal association between subunits of a native multi-subunit protein complex or the normal association of capsomeres in a native viral particle." Silva does not describe any method for *refolding* proteins from *non-native protein aggregates* at all, either with or without pressure cycling. Litt does not describe *refolding* of proteins from non-native protein aggregates without the use of pressure cycling. Accordingly, the "without repeatedly cycling the sample between the elevated and ambient pressures," element of Applicants' method is not taught by either Litt or Silva, alone or in combination. Therefore, the combination of the Litt and Silva references cannot render Applicants' claimed method obvious under Section 103(a).

Furthermore, the Silva reference examines dissociation and re-association of *native protein subunits* under pressure conditions that *do not denature* or completely dissociate the proteins, *i.e.*, pressures up to 2.5 kbar (Silva, page 15867, Col. 1). Silva does not directly address protein refolding, but does note that pressures over 2.5 kbar inhibited normal re-association of the dissociated protein subunits (Silva, page 15865, Fig. 3). Silva states that this is because very high pressures (*i.e.*, denaturing pressures) cause conformational changes (*i.e.*, unnatural folding) in the dissociated protein subunits that prevent their normal re-association and normal function (Silva, page 15868, col. 1). Silva also points out that similar results have been seen with subunits of other oligomeric proteins such as brome mosaic virus (Silva p. 15865, col. 2). Silva essentially teaches that Litt's method, which requires denaturing the dissociated proteins prior to refolding, does not work. Thus, Silva teaches against the method disclosed by Litt, which completely dissociates a protein aggregate and denatures the protein components before refolding (Litt, Col. 9, lines 15-24).

In view of these contradictory teachings, one of ordinary skill in the art would not have been motivated to combine the teachings of Silva, that *native* protein subunits dissociated at pressures that denature the subunits will not refold or re-associate normally, with the teachings

of Litt, that *non-native* protein aggregates must be denatured and subjected to pressure cycling in order to refold normally, to arrive at Applicants' claimed method of refolding proteins from *non-native* protein aggregates to their normal conformation in the absence of denaturation and pressure cycling.

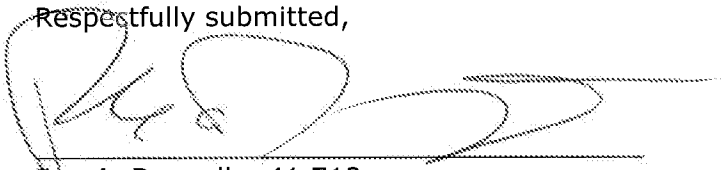
In addition, those of skill in the art would have had no reasonable expectation that non-denatured, disaggregated proteins would refold to their natural conformation in the absence of pressure cycling based on the combined teachings of Litt and Silva, because, even in combination, neither Litt nor Silva teaches or suggests the refolding of non-denatured proteins from non-native protein aggregates in the absence of pressure cycling.

For all of these reasons, Applicants respectfully request that the Section 103(a) rejection of claims 1-12 be withdrawn.

Conclusion

It is respectfully submitted that the claims are in condition for immediate allowance and a notice to this effect is solicited. The Examiner is invited to phone applicants' attorney if it is believed that a telephonic interview would expedite prosecution of the application.

Respectfully submitted,



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